IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| In re application of: Richard A. Berg | Art Unit: 1819 |
| Serial No.: 08/473,465 | Examiner: D. Clark |
| Filed: June 7, 1995 | Docket No.: C94-007D2 |
| Title: *Production of Human Recombinant Collagen in the Milk of Transgenic Animals* | |

DECLARATION UNDER RULE 132

I, Scott Leigh, declare and state as follows:

1. I am a research scientist at Cohesion Technologies, the assignee of the subject patent application. I am knowledgeable and experienced in collagen purification. I have read and am familiar with contents of the above application. My curriculum vitae is attached.

2. DEAE-cellulose ion exchange chromatography processes of separating and purifying individual collagen types from natural source collagen type mixtures, such as human fibroblasts, are inherently imperfect and result in a predominant type with small amounts of one or more other types.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application and any patent issuing therefrom.

Date: April 13, 99

Scott Leigh

# SCOTT LEIGH, Ph.D.

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## OBJECTIVE

A research, analytical, or process development position in the biotechnical industry performing basic or applied research.

## QUALIFICATIONS

Experience in assay development and characterization of recombinant proteins at Cohesion Technologies. Experience in the purification and characterization of bone growth factors and the development of immunoassays at Metra Biosystems. Experience in designing and conducting research in protein structure/function studies and cell biology studies with XOMA Corporation. Research and development experience in protein engineering with The Clorox Company in the development of enzymes for detergent applications. Extensive experience in protein purification, characterization, and assay development.

## PROFESSIONAL EXPERIENCE

**COHESION TECHNOLOGIES, INC. - COLLAGEN CORPORATION**, Palo Alto, California **Dec.1996-Present**

**Research Scientist III - Senior Scientist**

Research focuses on assay development and characterization of recombinant human collagen and other recombinant proteins. Responsibilities include leading an analytical development group, participation on project teams, and supervising research scientists, associates, and temporary employees. Assays are developed, validated, and protocols were written to support regulatory submissions and to provide support to other departments.

Techniques utilized include:

- protein chemistry - SDS gels, western blots, circular dichroism spectroscopy, differential scanning calorimetry, protein sequencing, amino acid analysis, and development of HPLC assays
- immunochemistry - generation of antibodies, screening, purification, and development of quantitative immunoassays using ELISA and automated formats

**METRA BIOSYSTEMS, INC.**, Mountain View, California **Feb. 1995-Dec. 1996**

**Research Scientist II (Research and Development)**

Research focused on purifying potentially novel bone growth factors and biochemical and biological characterization of the factors for use as potential therapeutics. The work involved purification techniques from the process level to analytical level separations. Growth factors were assayed and characterized by their ability to induce cell differentiation. Collagen degradation products were isolated and characterized as potential markers of bone metabolism.

Techniques utilized include:

- protein chemistry - tangential flow filtration and concentration; ion exchange, gel filtration, and hydrophobic interaction chromatography on both large and analytical scales; purification of modified amino acids, protein conjugations
- cell biology - activity assays and characterization of activities using cell culture systems

**XOMA CORPORATION**, Berkeley, California **1991-1995**

**Scientist II (Research Scientist, Biological Chemistry - PreClinical Research)**

Research involved the purification and characterization of recombinant proteins as well as native proteins from serum. Characterization of solution behavior, disulfide bonds, and investigating factors important for stability were performed to understand structure/function relationships. Cellular biology studies were performed investigating the binding, internalization, and trafficking of immunotoxins and monoclonal antibodies in human lymphoid cells. Managerial responsibilities included supervising and providing direction to a research scientist and a technical support person.

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## PROFESSIONAL EXPERIENCE (continued)

Techniques utilized include:

- protein chemistry - conjugations and covalent modifications of proteins (including monoclonal antibodies and F(ab) fragments), titration of sulfhydryls, photoaffinity labeling, determination of extinction coefficients, stability studies, turbidity measurements, peptide mapping, reverse-phase HPLC, peptide synthesis, formulation and preparation of proteins for *in vivo* studies, and analysis of pharmacokinetic samples
- protein purification - submilligram to multigram quantities of recombinant proteins, native proteins, or antibodies using ion-exchange, affinity, and gel filtration chromatography; filtration and tangential-flow concentration of large volume culture supernatants; assay development and adaptation of enzymatic assays to microtiter plate formats, ELISA and western assays, electrophoretic techniques
- cell biology - receptor/ligand characterization, radioiodination of proteins, binding assays, Scatchard analysis, immunoreactivity analysis, endocytosis measurements, cell fractionation, design of cytotoxicity assays

**THE CLOROX TECHNICAL CENTER**, Pleasanton, California 1988-1991

**Scientist II (Research Scientist, Biotechnology Group)**

Research involved the purification and characterization of novel proteases and lipases for study as laundry additives in detergent formulations. Purification protocols were developed for enzymes expressed and secreted by recombinant techniques as well as enzymes from their natural hosts. Protein sequencing was used to identify the N-terminal sequences of new enzymes and to provide information to design oligonucleotide probes. Autolytic properties of proteolytic enzymes were studied using HPLC techniques and autolytic sites were determined by protein sequencing. Peptide mapping was performed in order to identify residues involved in catalysis. The complete amino acid sequence was determined for a novel protease by protein sequencing and kinetic analysis was used to determine substrate specificities and sensitivities to inhibitors. Responsibilities included supervising the research of one other person, purchasing new equipment and basic maintenance and repair of equipment.

Techniques utilized included:

- protein/peptide purification - FPLC, peristaltic pump, and open-column techniques (both small scale and scale-up work) using ion exchange techniques and hydrophobic interaction chromatography; reverse-phase HPLC of peptides, peptide mapping, electrophoretic techniques
- sequencing/amino acid analysis - enzymatic and chemical digestion techniques, chemical modifications, solid-phase attachment chemistries, daily operation of protein sequencer, amino acid composition analysis using Pico-Tag chemistry
- enzyme kinetics - determination of kinetic constants for substrates and inhibitors

**DEPARTMENT OF CHEMISTRY AND BIOCHEMISTRY**, University of California, Los Angeles 1981-1988

**Research Assistant**

Dissertation title: *The Purification and Characterization of Rat Liver DNA Topoisomerase II and Topological Effects on Recombination in Mammalian Cells*

## EDUCATION

**Ph.D. in Biochemistry** - University of California, Los Angeles
Under the supervision of Dr. John M. Jordan

**B.S. in Biochemistry** - San Francisco State University

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# SCOTT LEIGH

## PUBLICATIONS

*Development of an Immunoassay for Urinary Galactosylhydroxylysine.* **Scott D Leigh**, Hsin-Shan Julia Ju, Robert Lundgard, George Y Daniloff, and Victor Liu. Journal of Immunological Methods, 220,169-178, (1998).

*Comparison of Analytical Performance and Biological Variability of Three Bone Resorption Assays.* Hsin-Shan Ju, Sunny Lung, Bradley Brown, Matthew A Stringer, **Scott Leigh**, Christy Scherrer, Karen Shepard, Dean Jenkins, Jane Knudsen, and Robert Cannon. Clinical Chemistry 43:9, 1570-1576 (1997).

*Biochemical Characterization of Recombinant Fusions of Lipopolysaccharide Binding Protein and Bactericidal/Permeability-Increasing Protein.* Susan L Abrahamson, Hsiu-Mei Wu, Robert E Williams, Ken Der, Nneka Ottah, Roger Little, Helene Gazzano-Santoro, Georgia Theofan, Robert Bauer, **Scott D Leigh**, Ann Orme, Arnold H Horwitz, Stephen F Carroll, and Russell L Dedrick. Journal of Biological Chemistry, 272, 2149-2155, (1997).

*Alteration of the Pharmacokinetics of Small Proteins by Iodination.* Robert J Bauer, **Scott D Leigh**, Cynthia A Birr, Susan L Bernhard, Maria Fang, Kenneth Der, Nneka Ottah Ihejeto, Stephen F Carroll, and Ada HC Kung. Biopharmaceutics & Drug Disposition, 17, 761-774, (1996).

*Expression and Characterization of Cysteine-Modified Forms of an Amino Terminal Fragment of Bactericidal-Permeability Increasing Protein.* Arnold H Horwitz, **Scott D Leigh**, Susan Abrahamson, Helene Gazzano-Santoro, Pei-Syan Liu, Robert E Williams, Stephen F Carroll, and Georgia Theofan. Protein Expression and Purification, 8, 28-40 (1996).

*T Cell-Targeted Immunofusion Proteins from Escherichia coli.* Marc Better, Susan L Bernhard, Robert E Williams, **Scott D Leigh**, Robert J Bauer, Ada HC Kung, Stephen F Carroll, and Diane M Fishwild. Journal of Biological Chemistry, 270, 14951-14957, (1995).

## PATENTS

*Collagen Peptide Assay Methods*
Patent Application, 1997
Inventors: Hsin-Shan Julia Ju, **Scott D Leigh**, Michael Byrne, Stephen Krane
Metra Biosystems

*Alkaline Serine Protease* Streptomyces Griseus *var.* Alkaliphus *Having Enhanced Stability Against Urea or Guanidine*
Inventor: **Scott Leigh**
Assignee: The Clorox Company
Patent Number: 5,646,028
Granted: July 8, 1997

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